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ANTIBIOGRAM AND VIRULENCE GENE DETECTION IN *ESCHERICHIA COLI* AND *VIBRIO* SPECIES ISOLATED FROM MARKET DUMPSITES IN EDO SOUTH SENATORIAL DISTRICT, NIGERIA

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ABSTRACT
Dumpsites are reported as a major source of human exposure to pathogenic microorganisms. In the current study, we investigated the prevalence of *Vibrio* sp. and *Escherichia coli* from market dumpsites in selected local government areas in Edo-South Senatorial district. Bacterial isolates were isolated using standard microbiological techniques, and further identified by their morphological, biochemical and 16 S rRNA sequence. The antibiotic susceptibility test and multidrug resistance profile were done by the agar disc diffusion method. DNA extraction was done by boiling method and the presence of virulent genes (*Ctx, TcpA$_{El\ tor}$, Zot, flicH7, Lt* and *Stx*) in the isolates were detected by polymerase chain reaction as well as agarose gel electrophoresis. *Vibrio cholerae* and *Escherichia coli* were prevalent in soil and leachates from market dumpsites in selected local government areas in Edo-South Senatorial district. All the isolates were multidrug resistant, while a total of 31 isolates (26 *Escherichia coli* and 5 *Vibrio* sp.) had at least, one of the virulent genes (*Lt, FliCH7, UidA, CtxA* and *TcpA$_{El\ tor}$*) of interest. This study shows that *Escherichia coli* and *Vibrio* sp. isolated from market dumpsites are multidrug resistant, and possesses virulent genes, thus, making them a threat to public health.

Keywords: Pollution; Environment; Public Health; Virulent genes; Dumpsites; Microorganisms.

INTRODUCTION

Waste is any solid or semisolid material which have been discarded, and may or may not be found useful by others, but could constitute nuisance to public health and the environment when not properly handled (Bartone, 2000; Buckle and Smith, 2000; Oluwande, 2002). Waste is classified as household, hazardous and biomedical, depending on their source. Household wastes are made of municipal wastes, construction and demolition debris, sanitation residue and waste from the streets. Residential and commercial complexes as well as markets generate the garbage that forms part of the wastes that is classified as household wastes. With the rising urbanization of Edo South Senatorial District and the change in lifestyles and food habits, the amounts of household wastes are on the increase (Adebayo, 1995; Aribisala *et al*., 2004). Hazardous wastes (mostly industrial) contain toxic substances that could be highly toxic to humans, animals and plants. They could be corrosive, highly inflammable or explosive and react when exposed to certain elements such as gases, old batteries, shoe polish, paint, old medicines, metal, chemicals, papers, pesticides, dyes, refining, rubber goods and medicine bottles (Adebayo, 1995; Aribisala, 1997). Biomedical wastes are highly infectious wastes or hospital wastes generated during the diagnosis, treatment or immunization of humans or animals or during research activities. They may include soiled wastes, disposables, anatomical wastes, discarded medicines, chemical wastes, etc which could be in form of disposable syringes, swabs, bandages, body fluids, human excreta, etc (Akpan, 1995). Waste management practices are not uniform among countries (developed and developing nations); regions (urban and rural areas), residential and industrial sectors because they all take different approaches (Davidson, 2011). Proper management of waste is important for building sustainable and livable cities, which includes the collection, transport, treatment and disposal of waste together with monitoring and regulation of the waste management processes (Davidson, 2011). Dumpsites are areas where waste materials are disposed and are viewed as the oldest form of waste management. Most waste dumpsites are located within the vicinity of living communities (Abdus-Salam *et al*., 2011).

Dumpsites in most developing countries remain a serious challenge, especially in big industrialized cities owing to indiscriminate dumping and littering of waste as well as delay in picking up such waste from dumpsites (Henry *et al*., 2006; Sharholy *et al*., 2008). This is worse when such waste is burnt (Guerrero *et al*., 2013), causing serious health and environmental problems (Al-Khatib *et al*., 2010). As reported by Kumar *et al*. (2009), more than 90% of waste in India is directly disposed on the land in an unsatisfactory manner and collection coverage is often lower than 60% (Henry *et al*., 2006; Zhang *et al*., 2010). Couth *et al*. (2011) reported that in Africa, emissions from waste dumpsites are 3 times higher than those in developed countries. Similarly, Guerrero *et al*. (2013) reported that waste management failure in developing countries is largely due to inadequacy of technical, environmental, financial, socio-cultural, institutional and legal aspects. Thus, waste management in developing countries is equated with land disposal or their discharge into drainage systems and water bodies (Cilinskis and Zaloksnis, 1996). This method of waste management is unscientific and constitutes a public health and environmental nuisance, resulting in pollution. When waste is dumped on land, the resulting microorganisms if pathogenic could pose a potential threat to public health. Open dumpsites are a potential source of microbial and toxic chemical pollution of the soil surrounding the dumpsites. This can also pollute hand dug wells posing serious threat to public health and leading to the destruction

of biodiversity in the environment (Ogunmodede *et al*., 2014). Water can also seep through the refuse pile in the dumpsites. This leads to the formation of leachates that are enriched in nutrients (nitrogen, potassium and phosphorous), heavy metals and other toxic substances including cyanide and dissolved organics (Ogbeibu *et al*., 2013). The composition of the wastes influences the concentration of the leachate constituents which may be absorbed on to the soil during this diffusion (Shaikh *et al*., 2012). This process creates health hazards, soil and water pollution and offensive odour which increase with an increase in ambient temperature levels (Abdus-Salam *et al*., 2011).

The aim of the current study was to determine the antimicrobial resistant profile and virulent gene detection in *Escherichia coli* and *Vibrio* sp. isolated from dumpsites in Edo South Senatorial District, Edo State, Nigeria.

MATERIALS AND METHODS

Sample Collection and Study Area

Soil samples and leachates (3-lots each) were obtained from 5 different markets in Edo South Senatorial District, Edo State, Nigeria. They included; Santana market dumpsite (Oredo Local Government Area), Iguobazuwa market dumpsite (Ovia-South West Local Government Area), Okada market dumpsite (Ovia-North East Local Government Area), Okha market dumpsite (Ikpoba Okha Local Government Area) and Uselu market dumpsite (Egor Local Government Area).

All samples were obtained in sterile polyethylene bag (for soil samples) and universal containers (for leachate) and transported to the Microbiology Laboratory of Benson Idahosa University in cold pack containing ice for immediate analysis.

Bacteriological Analysis

Sample Preparation

Serial dilutions of leachate and soil samples were carried out. For each sample, 10g of soil was dispensed into 90mL of sterile distilled water and homogenized, to form the stock solution. One millilitre of the stock solution was dispensed into 9mL of sterile distilled water in separate test tubes and mixed thoroughly making it a total of 10ml. The stock solutions were then serially diluted up to the ninth dilution (10^{-9}). Then 0.1mL of the appropriate dilution was cultured on nutrient agar, thio-citrate bile salt (TCBS) agar and eosin methylene blue (EMB) agar plates in triplicate, using the spread plate method and then incubated at 37˚C for 24h.

Isolation of *Vibrio* sp.

One milliliter each of the appropriate dilutions for both leachate and soil samples were cultured onto Thiosulphate citrate bile salt sucrose (TCBS) agar by the spread plate method and incubated at 37˚C for 24h. Distinct yellow colonies, characteristic of sucrose fermentation were sub-cultured on nutrient agar plates to obtain pure cultures. Pure cultures were then preserved on nutrient agar slants in bijou bottles for further studies.

Isolation of *Escherichia coli*

One milliliter each of the appropriate dilutions for both leachate and soil samples were cultured onto eosin methylene blue (EMB) agar by spread plate method and incubated at 37˚C for 24h. Distinct colonies characterized by greenish metallic sheen, with dark centres were sub-cultured on nutrient agar plates to obtain pure cultures. Pure cultures were then preserved on nutrient agar (double strength) slants in bijou bottles for further studies.

Identification of Bacterial Isolates

The bacterial isolates were identified using their cultural, morphological, biochemical as well as 16S rRNA analysis

Genomic DNA Extraction

Genomic DNA extraction was carried out by boiling method as described by Omoruyi *et al*. (2020). Briefly, isolates from stored slants were sub-cultured onto the appropriate medium, and incubated at 37˚C for 18h. Colonies of the fresh isolates were recovered into a 2mL Eppendorf tube containing 200µl of distilled water. Each suspension was thereafter vortexed for 2min. at maximum speed, using a vortex shaker, before being centrifuged for 10min. at 13,000rpm to break the cells. After centrifuging, 500µl of distilled water was added to the Eppendorf tube and vortexed for another 2min. The mixture was thereafter placed on a water bath at 100˚C for 15min. to finally release the DNA from the cell. The DNA was finally harvested by centrifugation at 10,000rpm for 5min. The DNA was harvested from the supernatant and stored at -20˚C in sterile Eppendorf tube for future use.

Antimicrobial Susceptibility Pattern

Antibiotics used

Selected *Vibrio* sp. and *Escherichia coli* were tested for their resistance and/or sensitivity against the following commonly used antibiotics: Augmentin (30µg), ceftazidime (30µg), cefuroxime (30µg), ciprofloxacin (5µg), gentamicin (10µg), ofloxacin (5µg), cefixime (5µg), ceftriaxone (30µg), erythromycin (5µg), cloxacillin (5µg) and nitrofurantoin (300µg). The antibiotics susceptibility pattern was determined according to the National Committee for Clinical Laboratory Standards (CLSI, 2014).

Standardization of Inoculum and Multi-Sensitivity Disc Test

Bacterial isolates were inoculated in peptone water and incubated at 37˚C for 24h. The broth was diluted using peptone water to obtain a density of 0.08-0.1 McFarland turbidity standard, using a spectrophotometer (Thermo Scientific GENESYS 10S UV-Vis Spectrophotometer). Sterile swab sticks were subsequently used to streak the diluted broth onto already prepared Muller Hilton agar plates within 30min. and allowed to dry for 5min. Sterile forceps was thereafter, used to place the antibiotics discs on top of the medium already impregnated with the bacterium. The plates were inverted and incubated aerobically at 37˚C for 24h. After incubation, the zones of inhibition were measured (in millimeters), using a meter ruler. All readings were interpreted according to the recommendation of the CLSI (2014).

Detection of Virulence Gene in *Vibrio* species

Following DNA extraction, the presence of three virulence genes (cholera toxin [*ctxA*], toxin-coregulated pilus [*tcpA Eltor*] and zonular occludence toxin [*zot*]) in all *Vibrio* species, were determined by polymerase chain reaction. The PCR was carried out using a thermocycler (Biometra, Gottingen, Germany) in a 25µl reaction containing 12.5µL of one Taq quick-load master mix (New England Biolabs, UK), 0.5µL of forward primer, 0.5µL of reverse primer, 1.5µL of template DNA and 10µL of nuclease-free water (AMRESCO, USA), with an initial denaturation temperature of 94˚C for 5min., followed by 39 cycles of amplicon denaturation at 94˚C

for 1min., primer annealing for 1min, elongation at 72˚C for 1min. and a final extension of 72˚C for 10min. All PCR products were given a holding temperature of 4˚C. The primers used, annealing temperatures as well as amplicon size are presented in Table 1.

Detection of Virulence Gene in *Escherichia coli* by Polymerase Chain Reaction

Following DNA extraction, the presence of four virulent genes (structural flagella antigen H7 [*FliCH7*], Beta-glucuronidase [*UidA*], heat-labile toxins [*Lt*] and shiga toxin [*Stx*]) in all *Escherichia coli*, were determined by polymerase chain reaction. The PCR was carried out using a thermocycler (Biometra, Gottingen, Germany) in a 25µl reaction containing 12.5µL of one Taq quick-load master mix (New England Biolabs, Inc.), 0.5µL of forward primer, 0.5µL of reverse primer, 1.5µL of template DNA and 10µL of nuclease free water (AMRESCO) with the conditions as shown in Table 1. All PCR products were given a holding temperature of 4°C.

Table 1: Primers used, PCR conditions and product size of the targeted genes.

Gene	Forward Primer (5' – 3')	Reverse Primer (3' – 5')	PCR Conditions	Product Size (bp)	Reference(s)
uidA	AAAACGGCAAGAAAAAGCAG	ACGCGTGGTTAACAGTCTTGCG	ID; 95°C for 2min, followed by 25 cycles of D; 94°C for 1min, A; 58°C for 1min, and E; 72°C for 1min, with a FE of 72°C for 2min	147	Alzubaidy (2019)
flicH7	TACCATCGCAAAAGCAACTCC	GTCGGCAACGTTAGTGATAC	ID; 95°C for 15min, followed by 35 cycles of D; 94°C for 45sec, A; 55°C for 45ec, and E; 68°C for 2min, with a FE of 72°C for 5min	230	Alzubaidy (2019)
Lt	GCACACGGAGCTCCTCAGTC	TCCTTCATCCTTTCAATGGCT	ID; 94°C for 5min, followed by 36 cycles of D; 94°C for 35sec, A; 62°C for 30ec, and E; 72°C for 1min, with a FE of 72°C for 5min	218	Alzubaidy (2019)
Stx	CAGTTAATGTGGTTGCGAAG	CTGCTAATAGTTCTGCGCATC	ID; 95°C for 2min, followed by 30 cycles of D; 94°C for 50sec, A; 55°C for 1min 30ec, and E; 72°C for 2min, with a FE of 72°C for 7min	895	Vargas *et al.* (1999)
Ctx	CTCAGACGGGATTTGTTAGGCACG	TCTATCTCTGTAGCCCCTATTACG	ID; 94°C for 5min, followed by 39 cycles of D; 94°C for 1min, A; 54°C for 1min, and E; 72°C for 1min, with a FE of 72°C for 10min	302	Abana *et al.* (2019)
tcpAElTor	GAAGAAGTTTGTAAAAGAAGAACAC	GAAAGGACCTTCTTTCACGTTG	ID; 94°C for 5min, followed by 39 cycles of D; 94°C for 1min, A; 54°C for 1min, and E; 72°C for 1min, with a FE of 72°C for 10min	618	Abana *et al.* (2019)
Zot	TCGCTTAACGATGGCGCGTTTT	AACCCCGTTTCACTTCTACCCA	ID; 94°C for 5min, followed by 39 cycles of D; 94°C for 1min, A; 54°C for 1min, and E; 72°C for 1min, with a FE of 72°C for 10min	947	Abana *et al.* (2019)

Key: ID: Initial Denaturation; D: Denaturation; A: Annealing; E: Extension; FE: Final Extension

Agarose Gel Electrophoresis

For *Escherichia coli*, the agarose gel was prepared by dissolving 1.5g of agarose in 100mL of one-time concentration (1x) of tris acetate ethylene diamine tetra-acetate (1x TAE) buffer and heating in a microwave for 2min. until the agarose was completely dissolved, while 1gram of agarose was weighed for *Vibrio* sp. For *Escherichia coli,* 0.3µL of ethidium bromide was added to the dissolved agarose solution as dye and mixed while for *Vibrio* sp., 0.2µL of ethidium bromide was added to the dissolved agarose solution as dye and mixed. The gel was then poured onto a mini horizontal gel electrophoresis well and the casting combs were arranged while filling the plasmid DNA. It was then allowed to set for 30min. The casting combs were carefully removed after the gel had completely solidified, one-time concentration (1x) of TAE electrophoresis buffer was then added to the reservoir until the buffer covered the agarose gel. Five microlitres (5µL) of gel loading dye (Bromophenol blue) was added to 25µL of each PCR product with gentle mixing. Ten microlitres (10µL) of the mixture was loaded onto the wells of the gel, the mini horizontal electrophoresis gel set up was covered and the electrodes connected running from cathode (-) to anode (+). Electrophoresis was carried out at 100mV for 45min. to allow easy separation of samples, based on their molecular weight. At the completion of the electrophoresis, the gel was removed from the buffer (Tris) and was viewed under a trans-illuminator UV light of wavelength 302nm. The band pattern of the DNA fragments was then photographed with a camera. Standard DNA molecular weight markers were used to estimate the plasmid size (100bp DNA ladder).

RESULTS

The results of the current study shows that enteric bacteria, particularly *Vibrio* species and *Escherichia coli* are prevalent in market dumpsites from selected local government areas in Edo South Senatorial District, Edo State, Nigeria. *Vibrio* species were present in soil samples obtained from all five local government areas investigated, which was also the case with leachate samples. Meanwhile, *E. coli* was present in 3 and 4 samples from soil and leachate respectively, leaving out Santana (Oredo) market, Okha (Ikpoba) market (Table 2). Table 3 shows the cultural, morphological and biochemical characteristics of the isolates. Forty (40) of the isolates were suspected to be *E. coli,* 31 as *Vibrio cholerae* and 9 as *Vibrio parahaemolyticus*. Following 16 S rRNA analyses, one *Escherichia coli* strain was confirmed to be *E. coli* O157:H7. The isolate have been deposited in the GenBank, and the accession number OM216847 assigned (Table 4). The pathogenic strain of *E. coli* was found in soil sample from Uselu market, Egor Local Government Area, Edo State.

All 40 *Escherichia coli* isolates were observed to be resistant to ceftazidime, cefuroxime, ceftriaxone, erythromycin, cloxacillin, Augmentin and cefixime (Table 5). Additionally, all the isolates were multidrug resistant, with only one isolate being resistant to 9 of the antibiotics used, while 35 of the isolates were resistant to at least 7 of the 11 antibiotics used (Table 6). All *Vibrio* species (n = 40) on the other hand were completely resistant to ceftazidime, cefuroxime, ceftriaxone, cloxacillin, Augmentin and cefixime, as well as sensitive to gentamycin (Table 7). The multidrug resistant profile further shows that eight of the isolates were resistant to at least 9 of the 11 antibiotics used, 4 were resistant to 8 of the

antibiotics, while all 40 isolates were multidrug resistant (Table 8). Table 9 shows the prevalence of 4 virulence genes (*FliCH7*, *UidA*, *Lt* and *Stx*) in all 40 *Escherichia coli* strains. Sixteen of the isolates had the *FliCH7* gene, 6 isolates had the *UidA* gene, 18 isolates had the *Lt* gene, while no isolate had the *Stx* gene. Only 10 of the 40 isolates had at least 2 virulent genes in them, while *Escherichia coli* O157:H7 had the *FliCH7*, *UidA* and *Lt* genes in it. Similarly, 18 *Vibrio cholerae* were screened for the presence and/or the absence of *CtxA*, *Zot*, and $TcpAEL_{tor}$ genes in them. Four of the isolates had *CtxA* gene, while only one isolate had the $TcpAEI_{tor}$ gene. Meanwhile, the *Zot* gene was completely lacking in all the isolates (Table 10).

Table 2: Prevalence of *Vibrio* species and *E. coli* from dumpsites located in major markets in Edo South Senatorial district

	Soil		**Leachate**	
	Vibrio spp.	*E. coli*	*Vibrio* sp.	*E. coli*
Santana (Oredo) Market Dumpsite				
Sample 1	+	-	+	-
Sample 2	+	-	+	+
Sample 3	+	+	+	+
Iguobazuwa (Ovia South-West) Market Dumpsite				
Sample 1	+	+	+	+
Sample 2	+	+	+	+
Sample 3	+	+	+	+
Okada (Ovia North-East) Market Dumpsite				
Sample 1	+	+	+	+
Sample 2	+	+	+	+
Sample 3	+	+	+	+
Ohka (Ikpoba-Ohka) Market Dumpsite				
Sample 1	+	+	+	-
Sample 2	+	+	+	-
Sample 3	+	+	+	+
Uselu (Egor) Market Dumpsite				
Sample 1	+	+	+	+
Sample 2	+	+	+	+
Sample 3	+	+	+	+

Key: +: present; -: absent; ND: Not determined

Table 3: Results of the Cultural, Morphological and Biochemical Characterization of Bacterial Isolates in Soil and Leachate from Market Dumpsites of Selected Markets in Edo South Senatorial District

Microscopic Examination & Gram Reaction	**Morphology**	**Biochemical Reactions**						**Presumptive Identity**	**Total Number of Isolates**
		Motility	**Citrate**	**Urease**	**Indole**	**Oxidase**	**Catalase**		
Gram negative short rods	Shiny green with black center and purity on EMB agar plate	+ve	-ve	-ve	+ve	-ve	+ve	*Escherichia coli*	40
Gram negative slightly curved rods	Yellow on TCBS agar plates	+ve	-ve	-ve	+ve	+ve	+ve	*Vibrio cholerae*	31
Gram negative short curved rods	Green on TCBS agar plate	+ve	+ve	+ve	+ve	+ve	+ve	*Vibrio parahaemolyticus*	9

Table 4: Sequence Analysis of 16S rRNA genes of Bacteria Isolated from Market Dumpsites in Edo South Senatorial District

Isolate Code	Closest match from GenBank	Query cover (%)	Percentage identity	E-Value	Accession Number
8-SEcE2	*E. coli* 0157:H7	98	95.98	0.0	OM216847

Key: SEcE2 - Soil *Escherichia coli* Egor

Table 5: Antibiotics susceptibility profile of *Escherichia coli* isolated from Leachate and Soil

Antibiotics	No. of isolates	% Resistance	% Sensitive	% Intermediate	Mean MDR Index
CAZ	40	100	-	-	100
CRX	40	100	-	-	100
GEN	40	18.2	63.6	18.2	18.2
CTR	40	100	-	-	100
ERY	40	100	-	-	100
CXC	40	100	-	-	100
OFL	40	-	72.8	27.2	-
AUG	40	100	-	-	100
CXM	40	100	-	-	100
NIT	40	36.4	45.5	18.2	36.4
CPR	40	27.2	54.6	18.2	27.2

KEY: CAZ = Ceftazidime; CRX = Cefuroxime; GEN = Gentamicin; CTR = Ceftriaxone; ERY = Erythromycin; CXC = Cloxacillin; OFL = Ofloxacin; AUG = Augmentin/Amoxicillin-clavulanate; NIT = Nitrofurantoin; CPR = Ciprofloxacin; CXM = Cefixime; I = Intermediate; R = Resistance; S = Sensitive; % = Number of antibiotics resistant x 100 divided by Number of antibiotics used

Table 6: MDR Profile of *Escherichia coli* Isolated from Leachate and Soil Dumpsites in Selected Markets in Edo-South Senatorial District

MDR profile									Bacterial isolates with MDR profile *Escherichia coli*	Total No. of isolates
CAZ	CRX	CTR	ERY	CXC	AUG	CXM			EEcONE1-4; EEcOSW1-4; EEclO1-4; EEcE1-4; EEcO3	17
CAZ	CRX	CTR	ERY	CXC	AUG	CXM			SEcONE1-2; SEcOSW1-4; SEclO1-4; SEcE1-4; SEcO1-4	18
CAZ	CRX	GEN	CTR	ERY	CXC	AUG	CXM		EEcO2; EEcO4	2
CAZ	CRX	CTR	ERY	CXC	AUG	CXM	NIT		SEcONE3-4	2
CAZ	CRX	GEN	CTR	ERY	CXC	AUG	CXM	CPR	EEcO1	1

Table 7: Antibiotics Susceptibility Profile of *Vibrio* sp. Isolated from Leachate and Soil of Market Dumpsites in Edo South Senatorial District

Antibiotics	No. of isolates	% Resistance	% Sensitive	% Intermediate	Mean MDR Index
CAZ	40	100	-	-	100
CRX	40	100	-	-	100
GEN	40	-	100	-	-
CTR	40	100	-	-	100
ERY	40	85	-	15	85
CXC	40	100	-	-	100
OFL	40	-	95.5	4.5	-
AUG	40	100	-	-	100
CXM	40	100	-	-	100
NIT	40	72.7	17.2	10.1	72.7
CPR	40	15	85	-	15

KEY: CAZ = Ceftazidime; CRX = Cefuroxime; GEN = Gentamicin; CTR = Ceftriaxone; ERY = Erythromycin; CXC = Cloxacillin; OFL = Ofloxacin; AUG = Augmentin/Amoxicillin-clavulanate; NIT = Nitrofurantoin; CPR = Ciprofloxacin; CXM = Cefixime; I = Intermediate; R = Resistance; S = Sensitive; % = Number of antibiotics resistant x 100 divided by Number of antibiotics used

Table 8: MDR Profile of *Vibrio* spp. Isolated from Leachate and Soil of Selected Market Dumpsites in Edo South Senatorial District

MDR profile									Bacterial isolates with MDR profile *Vibrio* spp.	Total No. of isolates
CAZ	CRX	CTR	CXC	AUG	CXM				SVsONE3; SVsE3; SVsO1	3
CAZ	CRX	CTR	CXC	AUG	CXM	NIT			EVslO1; EVsO1	2
CAZ	CRX	CTR	ERY	CXC	AUG	CXM			EVsONE1-4; EVsOSW1; EVsOSW3; EVslO2-4; EVsE1; EVsE3; EVsO2	12
CAZ	CRX	CTR	ERY	CXC	AUG	CXM			SVsOSW1-4; SVslO1; SVslO3; SVsE1-4; SVsO2	11
CAZ	CRX	CTR	ERY	CXC	AUG	CXM	NIT		EVsONE3	1
CAZ	CRX	CTR	CXC	AUG	CXM	NIT	CPR		EVsE2	1
CAZ	CRX	CTR	ERY	CXC	AUG	CXM	NIT		SVslO2	1
CAZ	CRX	CTR	CXC	AUG	CXM	NIT	CPR		SVsO3	1
CAZ	CRX	CTR	ERY	CXC	AUG	CXM	NIT	CPR	EVsOSW2; EVsOSW4; EVsO3-4; EVsO3; SVsONE1-4	4

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Table 9: Prevalence of *FliCH7*, *UidA*, *Lt* and *Stx* Genes in *Escherichia coli* Isolated From Market Dumpsites in Selected Local Government Area in Edo South Senatorial District

Virulence/Pathogenic Genes	Number of positive isolates (N = 40)	Number of negative isolates (N = 40)
FliCH7	16	24
UidA	6	34
Lt	18	22
Stx	0	40

Table 10: Prevalence of *CtxA*, *Zot* and *TcpAEl tor* Genes in *Vibrio cholerae* Isolated From Market Dumpsites in Selected Local Government Area in Edo South Senatorial District

Virulence/Pathogenic Genes	Number of positive isolates (N = 18)	Number of negative isolates (N = 18)
CtxA	4	14
Zot	0	18
TcpAELtor	1	17

DISCUSSION

Soil and leachate from waste dumpsites, particularly those obtained from markets, contribute significantly to the spread of antibiotic resistant bacteria, especially because a large part of the antibiotics, either used in livestock, food or consumed directly by humans eventually end up in dumpsites. *Vibrio cholerae* and *Escherichia coli* are some of the most predominant bacteria that are excreted from the human body, and their presence in dumpsites is an indication of its contamination, by waste of human and/or animal origin. The presence of theses isolates (*E. coli* and *Vibrio* species) in dumpsites is not unexpected, and has previously been reported by other studies (Idahosa *et al*., 2017; Nyandjou *et al*., 2018). People living closer to landfill sites are reported to suffer from several illnesses such as diarrhea, abdominal pain, cough, asthma, skin irritation, malaria, respiratory diseases, recurring flu, eye irritation, body weakness, cholera, tuberculosis etc. more than those living far away from landfill sites (Njoku *et al*. 2019; Daniel *et al*., 2021). It has also been reported that the location of dumpsites closer to sensitive areas such as market and residential buildings should be considered a public health threat (Daniel *et al*., 2021). Unclean water, dumpsites and stinky abattoirs were reported to have fueled a cholera outbreak in Bauchi state, Nigeria, in August, 2021 (Nigerian Guardian, 2021), killing 55 persons, with approximately 1,800 infected cases (Action Contre La Faim, 2021). The World Health Organization (2011) estimates that about a quarter of the diseases facing mankind today occur due to prolonged exposure to environmental pollution. The occurrences of *Escherichia coli* 0157:H7 and *Vibrio* spp. could be linked to its ability to withstand competition from the other indigenous microorganisms with higher growth rates (Lewis and Gattie, 2002). Municipal waste and contaminated bovine faeces are the most common type of waste present in dumpsites associated with *Escherichia coli* and *Vibrio* spp. (Aribisala *et al*, 2004). The ability of *Vibrio cholerae* to survive within and outside the aquatic environment makes cholera a complex health problem to manage (Osei and Duker, 2008). Cheesbrough (2006) reported that cholera is endemic in parts of Africa, South and South-East Asia where sanitation and hygiene are inadequate and clean water supplies are scarce.

The occurrence of these pathogenic Gram-negative bacteria in dumpsites suggest that there is a significant sanitary risk especially to the waste collectors, scavengers and those leaving in close proximity to the dumpsites.

Vibrio cholerae genotyping shows the level of relatedness of the strain and its importance in epidemiological studies. One of the *Vibrio* sp., was of the *tcpA El tor* biotype. *Vibrio* spp. O1 *El Tor* strains are known to have a better adaptability in the environment and are able to colonize more effectively in the intestinal lumen than the classical biotype (Ghosh-Banerjee *et al*., 2010).

The acquisition of a bacteriophage which encodes the *ctx* gene may be associated with the derivation of toxigenic *Vibrio* sp. strains from non-toxigenic progenitors (forbearers). Therefore, the higher the occurrence of the *ctx* gene, the more virulent or toxigenic the cholera outbreak will likely be. In this study, we identified 4 (22.2%) of the *ctx* gene in environmental isolates. The *zot* gene has been postulated to be present in toxigenic *V. cholerae* strains and likewise the *ctx* gene in *zot* positive strains (Ghosh *et al.*, 1997). However, contrary to these studies, we identified 4 (10%) of the isolates possessing the *ctx* gene without the *zot* gene while none of the *zot* gene was with or without the *ctx* gene for *V. cholerae* isolates, similar to a study conducted by Akoachere *et al*. (2014). These findings may suggest that the *zot* gene can occur independently of the *ctx* gene and as such can be used to explain the ability of some *V. cholerae* strains to cause illness in the absence of the cholera toxin. The absence of the *ctx* gene could be due to the *ctx*φ prophage genome (a filamentous phage whose single-stranded DNA genome includes *ctx*AB, the genes that encode cholera toxin (CT), the primary virulence factor produced by the choleragenic bacterium *Vibrio cholerae*) being missing or disrupted by mutations. In all, the predominant genotype among the *V. cholerae* isolates in this study were *Tcp El tor, ctx* and *zot*.

A review by Faruque *et al*. (2003) reported similar co-existence and possible gene transfer during co-existence of serogroups resulting in the emergence of novel pathogenic strains.

It has been shown that effective use of antibiotics reduces the duration of diarrhea, volume of stool losses by up to 50% and the duration of shedding of viable organisms in stool by patients from several days to 1–2 days (Folster *et al*., 2014). For *Escherichia coli* and *Vibrio* sp., eleven antibiotics recommended by the National Committee for Clinical Laboratory Standards (Augmentin/amoxicillin-clavulanate, ceftazidime, cefuroxime, ciprofloxacin, gentamicin, ofloxacin, cefixime, ceftriaxone,

erythromycin, cloxacillin and nitrofurantoin) (CLSI, 2014) were used in this study and revealed a loss of sensitivity to many of them which included ceftazidime, cefuroxime, cloxacillin, ceftriaxone, Augmentin/amoxicillin-clavulanate, cefixime and erythromycin which are used for first line treatment. Shrestha *et al*. (2015) reported 100% resistance of environmental isolates to erythromycin, with a comparable trend seen in South America (Goel and Jiang, 2010; Choudhry *et al.,* 2011). Ciprofloxacin, gentamicin and nitrofurantoin were found to be the most susceptible antimicrobial agents used against *E. coli* and *Vibrio* sp., similar findings have been reported in previous studies conducted in Addis Ababa, Ethiopia where ciprofloxacin, gentamicin and nitrofurantoin were susceptible among Gram negative bacteria isolated from pregnant women (Assefa *et al*., 2008).

Resistance to these antibiotics could be due to large scale abuse or extensive use of these antibiotics for the treatment of other infectious diseases other than for the treatment of cholera. It could also be due to the acquisition of the SXT element (an integrative and conjugative element (ICE) that acts as a vehicle for translocating many genes including the antibiotic resistance genes) (Waldor *et al*., 1996). The most important feature of SXT elements is that they carry multidrug resistant genes and transfer them between bacteria (Wang *et al*., 2016). The highest percentage of sensitivity of the *E. coli* isolates were recorded against ciprofloxacin, gentamicin, ofloxacin and nitrofurantoin while *Vibrio* sp. were gentamicin and ofloxacin as seen in other studies (Chomvarin *et al*., 2007; Goel and Jiang, 2010; ; Folster *et al*., 2014; Thapa Shrestha *et al*., 2015). The resistance pattern in the environmental isolates in this current study was not different from what was observed in the clinical isolates. It was however noted that none of *E. coli* isolates was resistant to ofloxacin and *Vibrio* sp. to gentamicin and ofloxacin

In our study, all the *Escherichia coli* isolates were *Stx* negative while all *Vibrio* sp. were *Zot* negative. The *fliCH7* gene is one of the genes used as a marker to identify EHEC O157:H7. Heat-labile (*Lt*) toxin encoding ETEC pathogroup was variously detected among the *E. coli* isolates. Of the 8 isolates harbouring the gene, 3 (7.5%) were from leachate and 5 (12.5%) from soil respectively. ETEC is the most important but under-recognized bacterial cause of diarrhea or cholera-like disease in all age groups especially in areas with population pressure, poor sanitation and inadequate drinking water (Kaper *et al*., 2004). It induces watery diarrhea in humans, distressing mostly infants and travelers (Turner *et al*., 2006). The strains aside being extensively responsible for millions of infection cases are significant pathogens linked to mortality from severe infantile diarrhea worldwide (Luo *et al*., 2014). The *Lt* gene was frequently detected among those recovered from surface waters of India and other South Asian countries polluted by human faeces (Begum *et al*., 2005; Ram *et al*., 2008). For the *UidA*, 6 (15%) *E. coli* gene were identified, 3 (50%) were from leachate and the other 3 (50%) from soil respectively, 7 (17.5%) possessing the *Lt* gene, and the *FliCH7* gene, 2 (5%) possessing the *FliCH7* gene and the *UidA* gene as well as 3 (7.5%) possessing *UidA* gene and the *Lt* genes.

The presence of pathogenic *E. coli* in the environment poses impending health dangers in animals and humans (Koczura *et al*., 2012). Several studies on incidences of virulence gene properties of *E. coli* have dealt extensively with isolates of clinical and environmental origin, thereby making it difficult to compare and contrast between our findings and previous investigations, especially soil and leachate. The spread of antibiotic resistance phenomena has drawn increasing attention in recent years because various infectious diseases that were once considered susceptible have steadily begun to be resistant to antimicrobial therapy, thus dashing man's hope in recovering from ailments (Titilawo *et al*., 2015). In the present study, antimicrobial resistance analysis reveals that all the strains were resistant to ceftazidime, cefuroxime, cloxacillin, ceftriaxone, Augmentin/amoxicillin-clavulanate, cefixime and erythromycin. Our findings concur with previous reports on the high level of resistance among isolates from leachate and soil (Chigor *et al*., 2010; Titilawo *et al*., 2015). Therefore, the occurrence of 80 enteric pathogenic bacteria in Edo South Senatorial District dumpsite is a matter of public health importance.

Conclusion

This study shows that multidrug resistant *E. coli* and *Vibrio* species possessing different virulent genes are prevalent in waste dumpsites (soil and leachates) from market dumpsites in selected Local Government Areas in Edo-South Senatorial district, hence, there is need to regularly dispose of market waste, to avoid any outbreak of diseases from piling of waste in market dumpsites.

Conflict Of Interest

The authors declare that there are no potential conflicts of interest

REFERENCES

Abana, D., Gyamfi, E., Dogbe, M., Opoku, G., Opare, D., Boateng, G. and Mosi, L. (2019). Investigating the virulence genes and antibiotic susceptibility patterns of *Vibrio cholerae* O1 in environmental and clinical isolates in Accra, Ghana. *BMC Infectious Diseases*. 19: 76-85.

Abdus-Salam, N., Ibrahim, M.S. and Fatoyinbo, F.T. (2011). Dumpsites in Lokoja, Nigeria. A silent pollution zone for underground water. *Journal of Waste Management and Bioresources Technology*, 3(1): 21-30.

Adebayo, W.O. (1995). Environmental Consequences of Waste Generation and Disposal Techniques along Roadsides, Drains and Stream Channels in a Tropical Urban center. *Environmental Issues*, 1(1): 1-4.

Akoachere, J. and Mbuntcha, C. (2014). Water sources as reservoirs of *Vibrio cholerae* O1 and non-O1 strains in Bepanda, Douala (Cameroon): relationship between isolation and physicochemical factors. *BioMed Central Infectious Diseases.* 14:421.

Akpan, E.A. (1995). Socio-Cultural and Economic Factors in Environmental Health, in *Environment and Health* (D) Olaniran, N.S., Akpan, E.A., Ikpeme, E.E. and Udofia, G.A. Nigeria Conservation Foundations Macmillan (Nig.) Publisher Limited Lagos. Pp. 157-174.

Al-Khatib, I. A., Monou, M., Zahra, A. S. F. A., Shaseen, H. Q. and Kassinos, D. (2010). Solid Waste characterization, quantification and management practices in developing countries. A case study: Nablus district Palestine. *Journal of Environmental Management*. 91:1131-1138.

Alzubaidy, Z.M. (2019). Detection of some virulence genes by PCR in *Escherichia coli* isolates from some local food in Erbil City. *Microbiology Research International*. 7(1): 1-9.

Aribisale, J. O. (1997). Waste Transport in Unsaturated Soils. *International Journal of Environment and Development*. Vol. 1, No. 1 pp. 49-54.

Aribisale, J. O., Omotoso, T. and Folorunso, P. (2004). Waste

Management Systems. Proceeding of National Civil Engineering Conference, Port Harcourt. Pp. 17-20.

Assefa, A., Asrat, D., Woldeamanuel, Y., Abdella, A. and Melesse, T. (2008). *Ethiopian Medical Journal*. 46: 227-35.

Bartone, C. R. (2000). Strategies for Improving Municipal Solid Waste Management: Lesson from a Decade of World Bank Landing. Regional conference. Partnership in Municipal Solid Waste Management Cairo, World Bank, Washington D. C. (Accessed October 2020).

Buckle and Smith (2000). Solid Waste Handling in Metropolitan. United States of America Public Health Service (USPHS) publication. Washington D. C. (Accessed October 2020).

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries, Part 1, 2 edition *Cambridge, UK: Cambridge University Press*. Pp. 97-115.

Chigor, V. N., Umoh, V. J. and Smith, S. I. (2010). Occurrence of *Escherichia coli* O157 in a river used for fresh produce irrigation in Nigeria. *African Journal of Biotechnology.* 9:178-182.

Chomvarin, C., Namwat, W., Wongwajana, S., Alam, M., Thaew-Nanngiew, K., Sichaturus, A. and Engchanil, C. (2007). Application of duplex-PCR in rapid and reliable detection of toxigenic *Vibrio cholerae* in water samples in Thailand. *Journal of General and Applied Microbiology*. 53 (4): 229-237.

Choudhry, S., Das, S., Saha, R., Ramachandran, V., Kaur, K. and Sarkar, B. (2011). Emergence of multiple drug resistance *Vibrio cholerae* O1 in East Delhi. *Journal of Infection in Developing Countries*. 15:104.

Cilinskis, E. and Zaloksnis, J. (1996). Solid Waste Management in the city of Riga, Latvia: Objectives and Strategy. *Ambio: A Journal of the Human Environment.* 25:103-107.

Clinical and Laboratory Standards Institute (CLSI). (2014). Performance standards for antimicrobial susceptibility testing. Twenty-Fourth Informational Supplement: *M100-S24*. 34(1).

Couth, R. and Trois, C. (2011). Waste management activities and carbon emissions in Africa. *Waste Management*. 31:131-137.

Davidson, G. (2011). Waste Management Practices. Dalhousie University-Office of Sustainability. (Accessed September 2020).

Faruque, S. M., Chowdhury, N., Kamruzzama, M., Rahman, M. H., Sack, D. A., Nair, G. B. and Mekalanos, J. J. (2003). Genetics diversity and virulence potential of environmental *Vibrio cholerae* population in a cholera-endemic area. *Proceeding of the National Academy of Sciences.* 101:2123-2128.

Folster, J., Katz, L., McCullough, A., Parsons, M., Knipe, K. and Sammons, S. (2014). Multidrug-resistant IncA/C plasmid in *Vibrio cholerae* from Haiti. *Emerging Infectious Diseases.* 20(11):1951–3.

Ghosh, C., Nandy, R., Dasgupta, S., Balakrish Nair, G., Hall, R. and Ghose, A. (1997). A search for cholera toxin (CT), toxin co-regulated pilus (TCP), the regulatory element ToxR and other virulence factors in non-O1/non-O139 *Vibrio cholerae. Microbial pathogenesis*. 22(4):199-208.

Ghosh-Banerjee, J., Senoh, M., Takahashi, T., Hamabata, T., Barman, S. and Koley, H. (2010). Cholera toxin production by the El Tor variant of *Vibrio cholerae* O1 compared to prototype *El Tor* and classical biotypes. *Journal of Clinical Microbiology*. 48(11):4283-6.

Goel, A. and Jiang, S. (2010). Genetic determinants of virulence, antibiogram and altered biotype among the *Vibrio cholerae* O1 isolates from different cholera outbreaks in India. *Infection, Genetics and Evolution.* 10(6):814-8.

Guerrero, L. A., Maas, G. and Hogland, W. (2013). Solid waste management challenges for cities in developing countries. *Waste Management*. 33:220-32.

Henry, R. K., Youngsheng, Z. and Jun, D. (2006). Municipal solid waste management challenges in developing countries Kenyan case study. *Waste Management.* 26:92-100.

Idahosa, I.B., Obueh, H.O., Aigbekaen, M.O. and Odesiri-Eruteyan, E.A. (2017). Characterization of potential pathogenic bacterial isolates from urban and rural market dumpsites. *International Journal of Microbiology and Biotechnology*. 2(3): 129-134.

Kaper, J. B., Nataro, J. P. and Mobley, H. L. (2004). Pathogenic *Escherichia coli. Natural and Reviewed Microbiology.* 2:123-140.

Koczura, R., Mokracka, J., Jabłonska, L., Gozdecka, E., Kubek, M. and Kazonowksi, A. (2012). Antimicrobial resistance of integron-harbouring *Escherichia coli* isolates from clinical samples, wastewater treatment plant and river water. *Science of the Total Environment*. 414:680–685.

Kumar, S., Bhattacharyya, J. K., Vaidya, A. N., Chakrabarti, T., Devotta, S. and Akolkar, A.B. (2009). *Waste Management.* 29:883-895.

Lewis, D. L. and Gattie, D. K. (2002). Pathogen risks from applying sewage sludge to land. *Environmental science Technology Journal*. 36: 286A-293.

Luo, Q., Kumar, P., Vickers, T. J., Sheikh, A., Lewis, W. G. and Rasko, D. A. (2014). Enterotoxigenic *Escherichia coli* secretes a highly conserved Mucin-degrading metalloprotease to effectively engage intestinal epithelial cells. *Infection and Immunity*. 82(2):509-521.

Miwanda, B., Moore, S., Muyembe, J., Nguefack-Tsague, G., Kabangwa, I. and Ndjakani, D. (2015). Antimicrobial drug resistance of *Vibrio cholerae*, Democratic Republic of the Congo. *Emerging Infectious Diseases*. 21(5):847–51.

Moyo, S. J., Gro, N. and Matee, M. I. (2011). Age specific aetiological agents of diarrhoea in hospitalized children aged less than five years in Dar es Salaam, Tanzania. *BMC Pediatrics*. Pp. 11:19.

Nyandjou, Y.M.C., Yakubu, S.E., Abdullahi, I.O. and Machido, D.A. (2018). Enteric bacteria of public health significance isolated from Zaria metropolis dumpsite soil. *Science World Journal*. 13(4): 30-34.

Ogbeibu, A. E., Chukwurah, A. E. and Oboh, I. P. (2013). Effects of open waste dump-site on its surrounding surface water quality in Ekurede-urhobo, Warri, Delta State, Nigeria. *Natural Environment*. 11: 1-16.

Ogunmodede, O. T., Adewole, E., Ajayi, O. O. and Onifade, A. K. (2014). Environmental assessment of solid waste management in Nigeria: a case study of Ikere Ekiti, Ekiti state. *Journal of Physical Chemistry and Science*. 11: 1-8.

Oluwande, P. A. (2002). An Overview of Urban Solid Waste Management in Nigeria. A paper presented at the Workshop on Waste Disposal Environmental Pollution and Community Health, Industrial unit and Technology. University of Ibadan. (Accessed October 2020).

Omoruyi, I.M., Obodo, I.C., Obukohwo, E.O. and Otoide, F.E. (2020). Virulent gene detection and antibiogramic profile of methicillin resistant *Staphylococcus aureus* isolated from birds of a poultry farm. *Ife Journal of Science*. 22(2): 45-55.

Osei, F. B. and Duker, A. A. (2008). Spatial dependency of *V. cholerae* prevalence on open space refuse dumps in Kumasi, Ghana: a spatial statistical modeling. *International Journal of Health Geography*. Pp. 7:62.

Shaikh, P. R., Bhosle, A. B. and Yannawar, V. B. (2012). The impact of landfill on soil and groundwater quality of the Nanded City, Maharashtra. 47: 56–63.

Sharholy, M., Ahmad, K., Mhamood, G., Trivedi, R. C. (2008). Municipal solid waste management in Indian Cities A review. *Waste Management*. 28:459-467.

Thepa Shrestha, U., Adhikari, N., Maharjan, R., Banjara, M., Rijal, K., Basnyat, S., and Agrawal, V. (2015). Multidrug resistant *Vibrio cholerae* O1 from clinical and environmental samples in Kathmandu city. *BMC Infectious Diseases*. 15(1):104.

Titilawo, O. Y., Obi, C. L. and Okoh, A. I. (2015). Occurrence of virulence gene signatures associated with diarrhoeagenic and non-diarrhoeagenic pathovars of *Escherichia coli* isolates from some selected rivers in South-Western Nigeria. *BMC Microbiology*. 15: 204.

Turner, S. M., Scott-Tucker, A., Cooper, L. M. and Henderson, I. R. (2006). Weapons of mass destruction: virulence factors of the global killer enterotoxigenic *Escherichia coli. Journal of the Federation of European Microbiological Societies (FEMS) Microbiology Letters*. 263:10-20.

Vargas, M., Gascon, J., De Anta, M.T.J. and Vila, J. (1999). Prevalence of *Shigella* enterotoxins 1 and 2 among *Shigella* strains isolated from patients with traveler's diarrhea. *Journal of Clinical Microbiology*. 37(11): 3608-3611.

Waldor, M. K., Tschape, H. and Mekalanos, J. J. (1996). A new type of conjugative transposon encodes resistance to sulfamethoxazole, trimethoprim, and streptomycin in *Vibrio cholerae* O139. *Journal of Bacteriology*. 178 4157-4165.

Wang, R., Yu, D., Yue, J. and Kan, B. (2016). Variations in SXT elements in epidemic *Vibrio cholerae* O1 *El Tor* strains in China. *Scientific Reports*. 6:22733.

World Health Organization (2011). Solid waste management in emergencies. Fact sheet No.7. (Accessed July 2020).

Zhang, C., Song, Y., Thompson, D. A., Madonna, M. A., Millhauser, G. L., Toro, S., Varga, Z., Westerfield, M., Gamse, J., Chen, W. and Cone, R. D. (2010). Proceedings of the National Academy of Sciences of the United States of America. 107(47): 20164-71.